

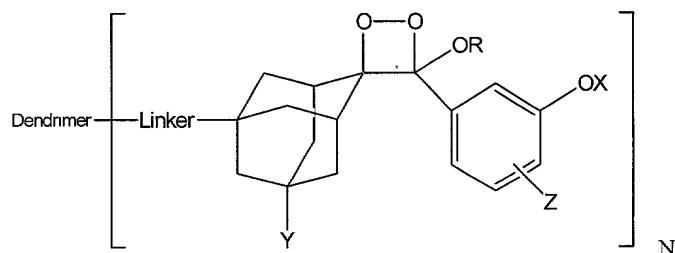
WHAT IS CLAIMED IS:

1. A chemiluminescent substrate delivery system comprising:
a dendrimer; and
at least one enzymatically activated chemiluminescent substrate conjugated
5 to the dendrimer.
2. The chemiluminescent substrate delivery system of Claim 1, wherein the chemiluminescent substrate comprises a moiety selected from the group consisting of a dioxetane moiety, a luminol moiety, an isoluminol moiety, an acridinium ester moiety, an acridinium sulfonylamine moiety, a luciferin moiety and combinations
10 thereof.
3. The chemiluminescent substrate delivery system of Claim 1, wherein a plurality of enzymatically activated chemiluminescent substrates are conjugated to the dendrimer.
4. The chemiluminescent substrate delivery system of Claim 3, wherein at
15 least two different enzymatically activated chemiluminescent substrates are conjugated to the dendrimer.
5. The chemiluminescent substrate delivery system of Claim 1, wherein the substrate delivery system is made by a method comprising:
covalently bonding one or more molecules comprising an enzymatically
20 activated chemiluminescent moiety to the dendrimer; or
covalently bonding one or more molecules comprising an enzymatically activated chemiluminescent moiety precursor to the dendrimer and thereafter
converting the chemiluminescent moiety precursor to a chemiluminescent moiety.

6. The chemiluminescent substrate delivery system of Claim 5, wherein
the dendrimer is selected from the group consisting of polyamidoamine dendrimers
with amino surface groups, polyamidoamine dendrimers with carboxylic acid
surface groups, polyamidoamine dendrimers with hydroxyl surface groups, and
5 polypropyleneimine dendrimers with amino surface groups.

7. The chemiluminescent substrate delivery system of Claim 1, wherein the
chemiluminescent substrate comprises a dioxetane moiety selected from the group
consisting of: 3-(2'-spiroadamantane) 4-methoxy-4-(3"-phosphoryloxy)phenyl-1,2-
dioxetane; disodium 3(4-methoxyspiro[1,2-dioxetane-3,2'-(5'-chloro)
10 tricyclo[3.3.1.1^{3,7}]decan]-4-yl)phenyl phosphate dioxetane; and disodium-2-chloro-
5-(4-methoxyspiro[1,2-dioxetane-3,2'-(5'-chloro-)tricyclo-{3.3.1.1^{3,7}}decan]-4yl)-
phenyl phosphate.

8. The chemiluminescent substrate delivery system of Claim 1, wherein the
chemiluminescent substrate comprises a dioxetane moiety and wherein the substrate
15 delivery system is represented by the formula:



wherein:

“Linker” represents a linker moiety;
20 “Dendrimer” represents a dendrimer moiety resulting from the reaction of a
surface functional group on the dendrimer with a functional group on the linker
moiety;

N is a positive integer representing the number of chemiluminescent substrates conjugated to the dendrimer moiety;

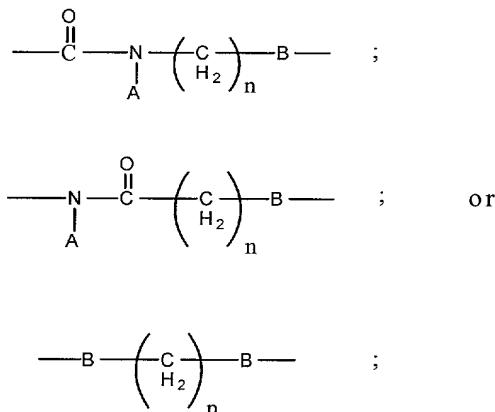
Y is H, a hydroxyl group, a halogen, an unsubstituted alkyl group, a hydroxy substituted alkyl group, a halogen substituted alkyl group, a phenyl group, a halogenated phenyl group, an alkoxy substituted phenyl group, an alkoxy phenoxy group, a hydroxy alkoxy group, a cyano group, an amide group, an alkoxy group or a carboxyl group;

R is a C₁ - C₁₂ alkyl, mono-, di-, or trihaloalkyl, an aryl or an aralkyl;

X is an enzyme-labile group selected from the group consisting of a phosphate, galactoside, acetate, 1-phospho-2,3-diacylglyceride, 1-thio-D-glucoside, adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, adenosine, α -D-glucoside, β -D-glucoside, β -D-glucuronide, β -D-mannoside, β -D-mannoside, β -D-fructofuranoside, β -glucosiduronate, 5-acetamido-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranoside, alkoxy derivatives of 5-acetamido-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranoside, p-toluenesulfonyl-L-arginine ester, and p-toluenesulfonyl-L-arginine amide; and

Z is a halo, alkoxy or alkyl group.

9. The chemiluminescent substrate delivery system of Claim 8, wherein the linker moiety is represented by the formula:



wherein:

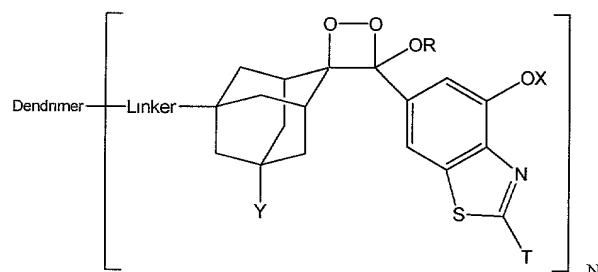
n is a positive integer;

A is H, alkyl, trihaloalkyl or aryl and

B is independently NA, NC(O)A, O, S or CH₂.

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10. The chemiluminescent substrate delivery system of Claim 1, wherein the chemiluminescent substrate comprises a dioxetane moiety and the delivery system is represented by the formula:



wherein:

“Linker” represents a linker moiety;

“Dendrimer” represents a dendrimer moiety resulting from the reaction of a surface functional group on the dendrimer with a functional group on the linker
5 moiety;

N is a positive integer representing the number of chemiluminescent substrates conjugated to the dendrimer moiety;

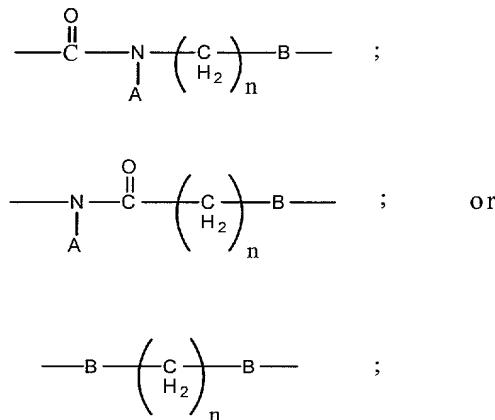
Y is H, a hydroxyl group, a halogen, an unsubstituted alkyl group, a hydroxy substituted alkyl group, a halogen substituted alkyl group, a phenyl group, a
10 halogenated phenyl group, an alkoxy substituted phenyl group, an alkoxy phenoxy group, a hydroxy alkoxy group, a cyano group, an amide group, an alkoxy group or a carboxyl group;

R is a C₁ - C₁₂ alkyl, a mono-, di-, or trihaloalkyl, an aryl group or an aralkyl group; and

15 X is an enzyme-labile group selected from the group consisting of a phosphate, galactoside, acetate, 1-phospho-2,3-diacylglyceride, 1-thio-D-glucoside, adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, adenosine, α -D-glucoside, β -D-glucoside, β -D-glucuronide, β -D-mannoside, β -D-mannoside, β -D-fructofuranoside, β -glucosiduronate, 5-acetamido-3,5-dideoxy- α -
20 D-glycero-D-galacto-2-nonulopyranoside, alkoxy derivatives of 5-acetamido-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranoside, p-toluenesulfonyl-L-arginine ester, and p-toluenesulfonyl-L-arginine amide; and

T is H, an electron donating group, an electron withdrawing group, or an organic linker group which may be attached to an ancillary fluorophore or to any biological moiety.

11. The chemiluminescent substrate delivery system of Claim 10, wherein
5 the linker moiety is represented by the formula:



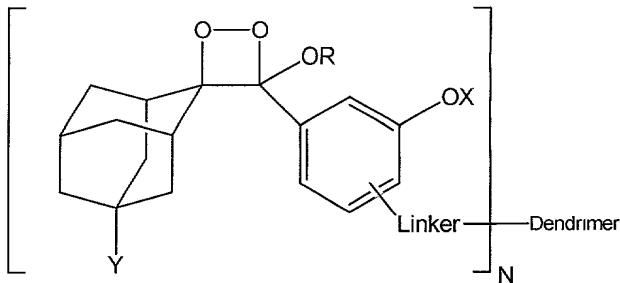
wherein:

n is a positive integer;

A is H, alkyl, trihaloalkyl or aryl; and

B is independently NA, NC(O)A, O, S or CH₂.

10 12. The chemiluminescent substrate delivery system of Claim 1, wherein
the chemiluminescent substrate comprises a dioxetane moiety and the delivery
system is represented by the formula:



wherein:

“Linker” represents a linker moiety;

“Dendrimer” represents a dendrimer moiety resulting from the reaction of a 5 terminal functional group on the dendrimer with a functional group on the linker moiety;

N is a positive integer representing the number of chemiluminescent substrates conjugated to the dendrimer moiety;

Y is H, a hydroxyl group, a halogen, an unsubstituted alkyl group, a hydroxy 10 substituted alkyl group, a halogen substituted alkyl group, a phenyl group, a halogenated phenyl group, an alkoxy substituted phenyl group, an alkoxy phenoxy group, a hydroxy alkoxy group, a cyano group, an amide group, an alkoxy group or a carboxyl group;

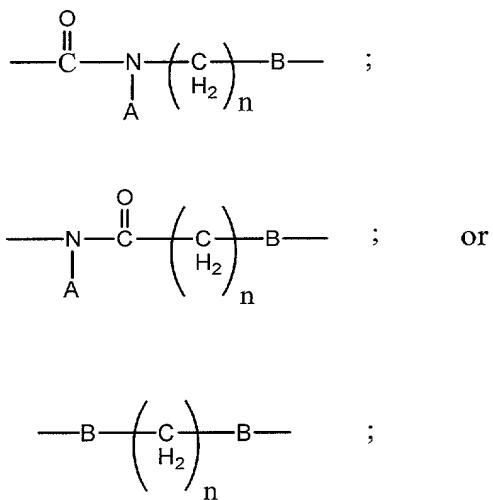
R is a C₁ - C₁₂ alkyl, mono-, di-, or trihaloalkyl, an aryl or an aralkyl; and

15 X is an enzyme-labile group selected from the group consisting of a phosphate, galactoside, acetate, 1-phospho-2,3-diacylglyceride, 1-thio-D-glucoside, adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, adenosine, α -D-glucoside, β -D-glucoside, β -D-glucuronide, β -D-mannoside, β -D-mannoside, β -D-fructofuranoside, β -glucosiduronate, 5-acetamido-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranoside, alkoxy derivatives of 5-acetamido-3,5-

dideoxy- α -D-glycero-D-galacto-2-nonulopyranoside, p-toluenesulfonyl-L-arginine ester, and p-toluenesulfonyl-L-arginine amide.

13. The chemiluminescent substrate delivery system of Claim 12, wherein the linker moiety is represented by the formula:

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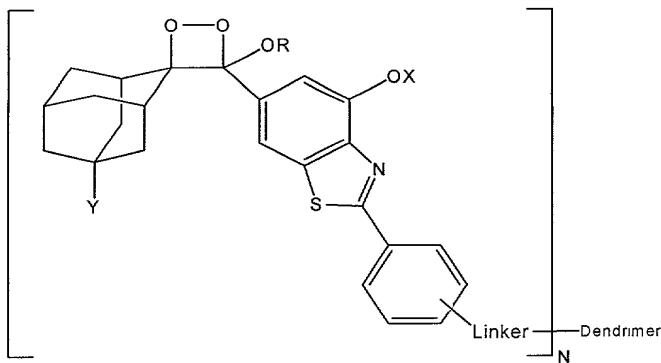
wherein:

10 n is a positive integer;

 A is H, alkyl, trihaloalkyl or aryl; and

 B is independently NA, NC(O)A, O, S or CH₂.

14. The chemiluminescent substrate delivery system of Claim 1, wherein the chemiluminescent substrate comprises a dioxetane moiety and the delivery system is represented by the formula:



wherein:

“Linker” represents a linker moiety;

“Dendrimer” represents a dendrimer moiety resulting from the reaction of a
5 terminal functional group on the dendrimer with a functional group on the linker
moiety;

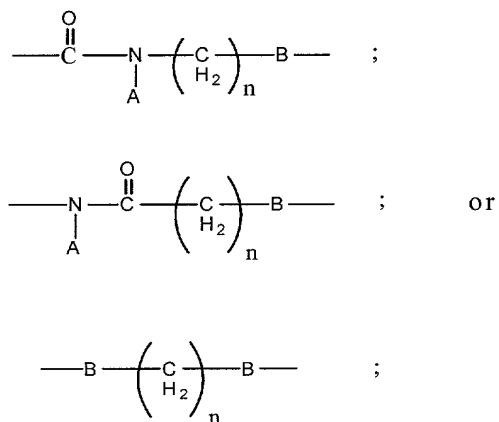
N is a positive integer representing the number of chemiluminescent
substrates conjugated to the dendrimer moiety;

Y is H, a hydroxyl group, a halogen, an unsubstituted alkyl group, a hydroxy
10 substituted alkyl group, a halogen substituted alkyl group, a phenyl group, a
halogenated phenyl group, an alkoxy substituted phenyl group, an alkoxy phenoxy
group, a hydroxy alkoxy group, a cyano group, an amide group, an alkoxy group or
a carboxyl group;

R is a C₁ - C₁₂ alkyl, mono-, di-, or trihaloalkyl, an aryl or an aralkyl; and
15 X is an enzyme-labile group selected from the group consisting of a
phosphate, galactoside, acetate, 1-phospho-2,3-diacylglyceride, 1-thio-D-glucoside,
adenosine triphosphate, adenosine diphosphate, adenosine monophosphate,
adenosine, α -D-glucoside, β -D-glucoside, β -D-glucuronide, β -D-mannoside, β -D-
mannoside, β -D-fructofuranoside, β -glucosiduronate, 5-acetamido-3,5-dideoxy- α -

D-glycero-D-galacto-2-nonulopyranoside, alkoxy derivatives of 5-acetamido-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranoside, p-toluenesulfonyl-L-arginine ester, and p-toluenesulfonyl-L-arginine amide.

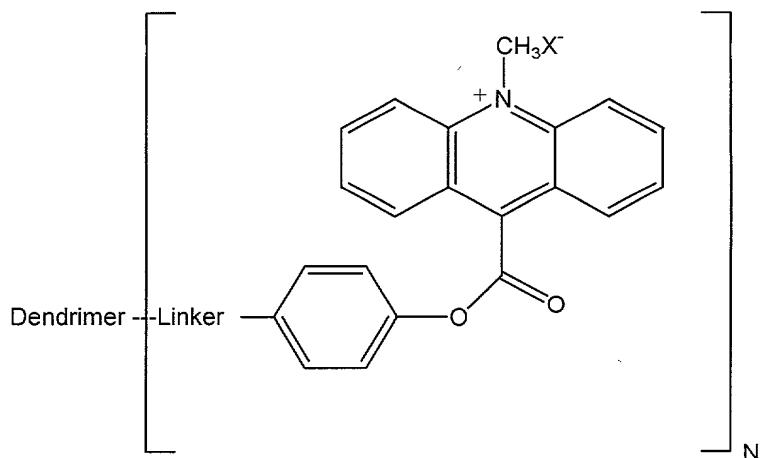
15. The chemiluminescent substrate delivery system of Claim 14, wherein
5 the linker moiety is represented by the formula:



wherein:

10 n is a positive integer;
A is H, alkyl, trihaloalkyl or aryl; and
B is independently NA, NC(O)A, O, S or CH₂.

16. The chemiluminescent substrate delivery system of Claim 1, wherein
the chemiluminescent substrate comprises an isoluminol moiety and the delivery
15 system is represented by the formula:



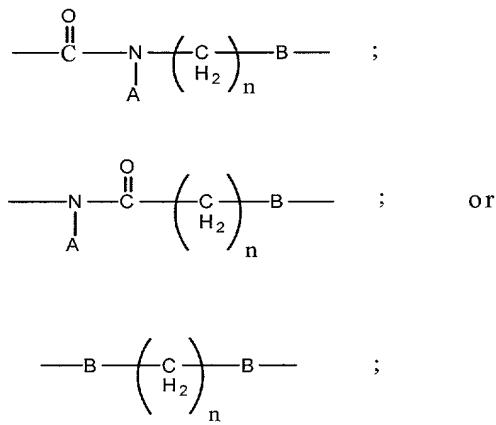
wherein:

“Linker” represents a linker moiety;

“Dendrimer” represents a dendrimer moiety resulting from the reaction of a
 5 surface functional group on the dendrimer with a functional group on the linker
 moiety; and

N is a positive integer representing the number of chemiluminescent
 substrates conjugated to the dendrimer moiety.

17. The chemiluminescent substrate delivery system of Claim 16, wherein
 10 the linker moiety is represented by the formula:



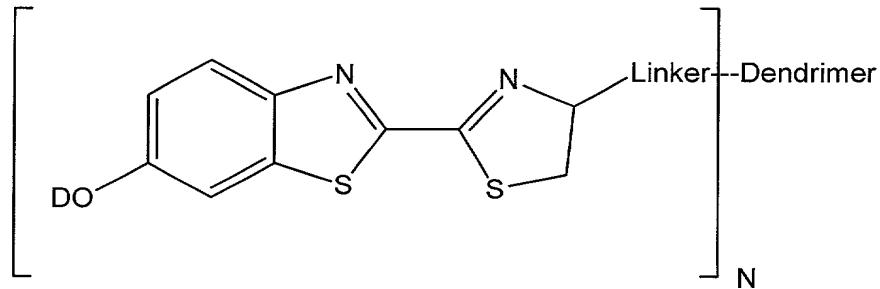
wherein:

n is a positive integer;

A is H, an alkyl group, a trihaloalkyl group or an aryl group; and

B is independently NA, NC(O)A, O, S or CH₂.

5 18. The chemiluminescent substrate delivery system of Claim 1, wherein
the chemiluminescent substrate comprises luciferin moiety and the delivery system
is represented by the formula:



wherein:

“Linker” represents a linker moiety;

10 “Dendrimer” represents a dendrimer moiety resulting from the reaction of a
surface functional group on the dendrimer with a functional group on the linker
moiety;

N is a positive integer representing the number of chemiluminescent
substrates conjugated to the dendrimer moiety; and

15 D is hydrogen or an enzyme-labile group selected from the group consisting
of phosphate, galactoside, acetate, 1-phospho-2,3-diacylglyceride, 1-thio-D-
glucoside, adenosine triphosphate, adenosine diphosphate, adenosine
monophosphate, adenosine, α -D-glucoside, β -D-glucoside, β -D-glucuronide, β -D-
mannoside, β -D-mannoside, β -D-fructofuranoside, β -glucosiduronate, 5-acetamido-

3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranoside, alkoxy derivatives of 5-acetamido-3,5-dideoxy- α -D-glycero-D-galacto-2-nonulopyranoside, p-toluenesulfonyl-L-arginine ester, and p-toluenesulfonyl-L-arginine amide a PO_3H_2 group.

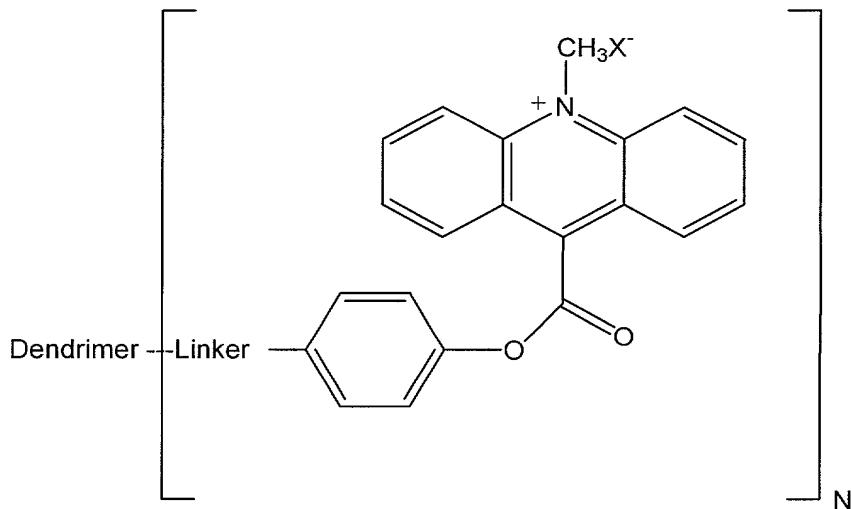
5 19. The chemiluminescent substrate delivery system of Claim 18, wherein the linker moiety is represented by:

--COO(CH₂)_n-NA-C(O)--;
--COO(CH₂)_n-C(O)-NH--; or
--COO(CH₂)_n-B--;

10 wherein:

n is a positive integer;
A is hydrogen or an alkyl group; and
B is NA, O, S or CH₂.

20. The chemiluminescent substrate delivery system of Claim 1, wherein
15 the chemiluminescent substrate comprises an acridinium ester moiety and the delivery system is represented by the formula:



wherein:

“Linker” represents a linker moiety;

“Dendrimer” represents a dendrimer residue resulting from the reaction of a surface functional group on the dendrimer with a functional group on the linker
5 moiety;

N is a positive integer representing the number of chemiluminescent substrates conjugated to the dendrimer moiety; and

X is a counterion.

21. The chemiluminescent substrate delivery system of Claim 20, wherein
10 the linker moiety is represented by:

--C(O)-NA-(CH₂)_n--;

--NH-C(O)-(CH₂)_n--; or

--B-(CH₂)_n--;

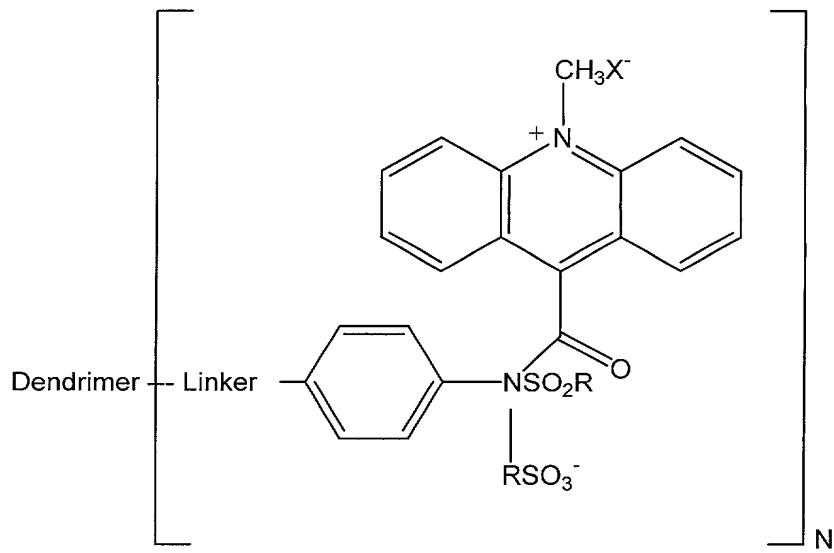
wherein:

15 n is a positive integer;

A is hydrogen, an alkyl group or an aryl group; and

B is NA, O, S or CH₂.

22. The chemiluminescent substrate delivery system of Claim 1, wherein
the chemiluminescent substrate comprises an acridinium sulfonyl amide moiety and
20 the delivery system is represented by the formula:



wherein:

“Linker” represents a linker moiety;

5 “Dendrimer” represents a dendrimer moiety resulting from the reaction of a surface functional group on the dendrimer with a functional group on the linker moiety;

N is a positive integer representing the number of chemiluminescent substrates conjugated to the dendrimer moiety;

10 R is an alkyl or an aryl group; and

X is a counterion.

23. The chemiluminescent substrate delivery system of Claim 22, wherein the linker moiety is represented by:

--C(O)-NA-(CH₂)_n--;

15 --NH-C(O)-(CH₂)_n--;

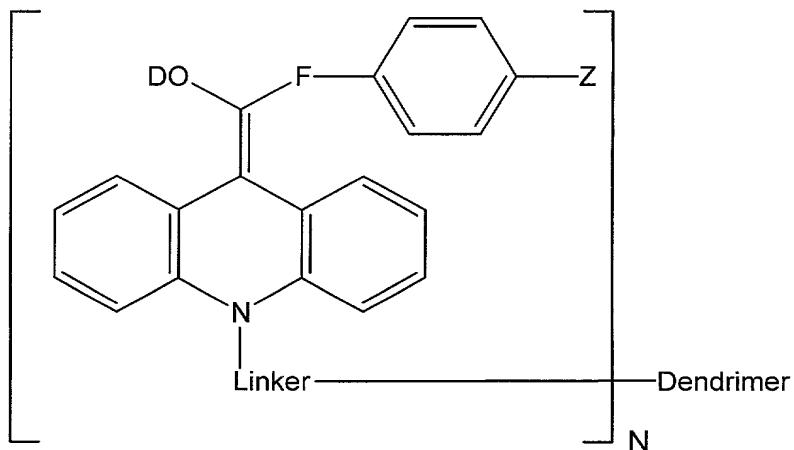
--B-(CH₂)_n--;

--C(O)-NA-(CH₂)_n-C(O)NH--;
--NH-C(O)-(CH₂)_n-C(O)NH--; or
--B-(CH₂)_n-C(O)NH--;

wherein:

5 n is a positive integer;
A is hydrogen, an alkyl group or an aryl group; and
B is NA, O, S or CH₂,

24. The chemiluminescent substrate delivery system of Claim 1, wherein
the chemiluminescent substrate comprises an acridan moiety and the delivery
10 system is represented by the formula:



wherein:

15 “Linker” represents a linker moiety;
“Dendrimer” represents a dendrimer moiety resulting from the reaction of a
surface functional group on the dendrimer with a functional group on the linker
moiety;
N is a positive integer representing the number of chemiluminescent
20 substrates conjugated to the dendrimer moiety;

D is a PO_3X_2 group, a glycoside or a sulfate wherein X is a counterion;

F is NA, S or O; and

Z is a halo, alkoxy or alkyl group.

25. The chemiluminescent substrate delivery system of Claim 24, wherein

5 the linker moiety is represented by:

-- $(\text{CH}_2)_n\text{NA-C(O)-}$;

-- $(\text{CH}_2)_n\text{-C(O)-NA-}$; or

-- $(\text{CH}_2)_n\text{-B-}$;

wherein:

10 n is a positive integer;

A is hydrogen or an alkyl group; and

B is NA, O, S or CH_2 .

26. The chemiluminescent substrate delivery system of Claim 1, wherein

the dendrimer is covalently or ionically associated with one or more additional

15 dendrimers.

27. The chemiluminescent substrate delivery system of Claim 1, wherein
the substrate delivery system further comprises a chemiluminescence enhancer
moiety.

28. The chemiluminescent substrate delivery system of Claim 27, wherein
20 the enhancer moiety is formed by coupling a chemiluminescent enhancing molecule
to a reactive site on the dendrimer or by chemically modifying a reactive site on the
dendrimer to an enhancing moiety.

29. The chemiluminescent substrate delivery system of Claim 28, wherein
the enhancer moiety is formed by peralkylation of amino groups on the dendrimer or

by peralkylcarbonylation of amino groupds on the dendrimer by alkylation of amide groups on the dendrimer.

30. The chemiluminescent substrate delivery system of Claim 28, wherein the enhancer moiety is formed by reaction of carboxylate groups on the dendrimer 5 with an amino linked ammonium, phosphonium or sulfonium salt.

31. The chemiluminescent substrate delivery system of Claim 1, further comprising a second dendrimer, wherein the second dendrimer comprises a chemiluminescence enhancer moiety and wherein the second dendrimer is covalently or ionically associated with the dendrimer conjugated to the at least one 10 enzymatically active chemiluminescent substrate moiety.

32. The chemiluminescent substrate delivery system of Claim 1, wherein the chemiluminescent substrate and/or the dendrimer comprises one or more water solubilizing groups.

33. The chemiluminescent substrate delivery system of Claim 32, wherein 15 the one or more water solubilizing groups are selected from the group consisting of carboxylic acids, esters, alkyl-oxides, aryl-oxides, alkyl-amides, aryl-amides, aralkyl-amides, alkyl-urethanes, aryl-urethanes, alkyl-sulfonamides, aryl-sulfonamides, alkyl-sulfonic acids, aryl-sulfonic acids, quaternary ammonium salts, and combinations thereof.

20 34. A kit for conducting an assay to determine the presence or concentration of an analyte in a sample, wherein the kit comprises:

a dendrimer comprising at least one enzymatically active chemiluminescent substrate moiety; and

an enzyme capable of activating the chemiluminescent substrate to produce a peroxygenated intermediate that decomposes to produce light.

35. The kit of Claim 34, wherein the enzymatically activated chemiluminescent substrate moiety comprises a dioxetane having an enzyme labile group and wherein the enzyme is capable of cleaving the enzyme labile group to produce the peroxygenated intermediate.

36. The kit of Claim 35, further comprising a chemiluminescent enhancement substance.

37. The kit of Claim 34, wherein the assay is an immunoassay and the 10 enzyme is complexed with an agent capable of binding to the analyte.

38. The kit of Claim 34, wherein the assay is a DNA probe assay, the kit further comprising a membrane on which the assay can be conducted.

39. The kit of Claim 38, further comprising an enhancement substance for increasing the chemiluminescent signal obtained from the substrate.

15 40. The kit of Claim 38, wherein the enzyme is complexed with an agent and wherein the agent can form a complex with the analyte.

41. The kit of Claim 34, wherein the assay is a DNA sequence analysis assay and the kit further comprises a membrane on which said sequence analysis assay can be conducted.

20 42. The kit of Claim 41, wherein the kit further comprises a chemiluminescent enhancement substance.

43. The kit of Claim 41, wherein the enzyme is complexed with an agent permitting attachment of the enzyme to the DNA to be sequenced in the assay.

44. The kit of Claim 34, wherein the enzyme is capable of oxidizing the chemiluminescent substrate to produce the peroxygenated intermediate.

45. The kit of Claim 44, wherein the chemiluminescent substrate comprises a moiety selected from the group consisting of a luminol moiety, an isoluminol moiety, an acridinium ester moiety, an acridinium sulfonylamide moiety or a luciferin moiety.

46. A method for detecting the presence of an analyte in a sample comprising:

10 forming an enzyme complex between an enzyme and a substance capable of binding to the analyte;

15 adding the enzyme complex to the sample;

allowing the enzyme complex to bind with analyte present in the sample;

adding a chemiluminescent delivery system to the sample; and

measuring chemiluminescent emissions from the sample;

wherein the chemiluminescent delivery system comprises a dendrimer and at least one enzymatically active chemiluminescent substrate conjugated to the dendrimer and wherein the amount of chemiluminescent emissions measured indicates the presence and/or the concentration of the analyte in the sample.

47. The method of Claim 46, further comprising a step of removing enzyme complex which did not bind with the analyte prior to the measuring step.

48. The method of Claim 46, wherein the substance capable of binding to the analyte is an antigen, an antibody or a nucleic acid probe.

49. The method of Claim 46, wherein the assay is conducted on a solid support.

50. The method of Claim 49, wherein the presence of an analyte is detected in a plurality of samples arranged in an array on the solid support.

51. The method of Claim 49, further comprising binding the analyte to the solid support.

52. The method of Claim 46, wherein the enzyme is selected from the group consisting of glycosidases, esterases, proteases, oxidases, peptidases and phosphatases.